Histidine phosphatases and histidine kinases are enzymes acting in opposite directions. Histidine kinase effects the phosphorylation of certain histidine residues in proteins, whereas histidine phosphatases reverse this phosphorylation. Both enzymes probably play an important part in signal transduction, apoptosis, the control of cell growth and in cell differentiation. They are known to be diseases based on disturbances of these cellular functions. The present invention is thus based on the object of providing an agent which can be used to investigate the cause of the pathophysiological disturbances and, where appropriate, treat them.

Hormones or peptides stimulate cell surface receptors on a cell and induce cellular effects via a signal transduction pathway. Reversible phosphorylation of specific protein substrates by regulatory protein kinases and phosphatases plays an essential part in intracellular signal transmission. Receptor-bound, membrane-bound and intracellular protein kinases and phosphatases regulate the processes of cellular proliferation, cell differentiation and the immune system. Malfunctioning of these regulators or their activities is crucial for a large number of pathophysiological effects. Accordingly, protein kinases and phosphatases and the signal transduction pathway in which they are involved represent potential targets for the drug discovery process.

Signal transduction in mammals involves reversible phosphorylation of Ser/Thr/Tyr. Although histidine phosphate is known to be present in mammals (Crovello CS, Furie BC, Furie B (1995) Cell 82:279-286), it has not to date been possible to identify either the corresponding kinases or the relevant phosphatases. The difficulty is, inter alia, that histidine phosphate is unstable to hydrolysis and is not detected in standard phospho amino acid analysis.

The functions of His kinases and His phosphatases in bacteria have been investigated very thoroughly. Their involvement in chemotaxis and adaptation makes them promising points of attack for bacterial diseases.

Summary of the Invention

The present invention relates to histidine protein phosphatase, in particular histidine protein phosphatase polypeptides and histidine protein phosphatase polynucleotides, recombinant materials and methods for their production. Such polypeptides and polynucleotides are of interest in relation to methods of treatment of certain diseases, including, but not limited to, cancer and metabolic disorders, cardiovascular diseases and diseases of the central nervous system, hereinafter referred to as "diseases of the invention". In a further aspect, the invention relates to methods for identifying agonists and antagonists (e.g., inhibitors) using the materials provided by the invention, and treating conditions associated with N-phosphorylation imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate histidine protein phosphatase activity or levels.

The invention likewise includes corresponding variants and mutants, produced, for example, by random or controlled substitution, different splicing, deletion or addition of one or more nucleotides or amino acids, with the biological activity being essentially retained.

Thus, it is an object of the present invention to provide a polypeptide having the biological activity of a histidine phosphatase which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000, obtainable by purification from mammalian tissue by at least one anion exchange chromatography, one gel filtration and one affinity chromatograph step. Preferred mammalian tissue is derived from heart, kidney, liver, pancreas, skeletal muscle and testis. Preferred mammals are humans, rabbits, rats.

The polypeptide of the invention comprises at least the amino acid sequence motif (SEQ. No. 3) DCECLGGGRISHQSQD.

In another embodiment of the invention the polypeptide comprises at least the amino acid sequence motif (SEQ. No. 4)

DCECLGGGRISHQSQDX^{1}KIHVYGYSMX^{2}YGX^{3}AQH

wherein X^1 = K or R, X^2 = A or G and X^3 = P or R.

In a further embodiment of the invention the polypeptide comprises at least the amino acid sequence motif (SEQ. No. 5)

YHADIYDKVSGDMQKQGCDCECLGGGRISHQSQDKKIHVYGYSM.

All these partial sequences are highly conserved within the complete enzyme amino acid sequence and are deemed to be involved in the active site of said enzyme or have other biological or pharmaceutical relevance in mammals.

As a preferred embodiment of the invention the polypeptide has the biological activity of a histidine phosphatase which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000 and comprises the following amino acid sequence (SEQ. No. 2):

(M)AVADLALIPDVDIDSDGVFKYVLIRVHSAPRSGAPAAESKEIVRGYKWAEYHADIYDKVSGD
MQKQGCDCECLGGGRISHQSQDKKIHVYGYSMAYGPAQHAISTEKIKAKYPDYEVTWANDGY

The methionine residue at the N-terminal of the sequence is not obligatory. The above indicated amino acid sequence is of human origin.

However, the invention discloses also especially homologue variants of said sequences. Therefore, it is a further object of the invention to provide a polypeptide having the biological activity of a histidine phosphatase which has a high specificity for phosphohistidine and a molecular weight of 13.000 –15.000, the amino acid sequence of which has a homology of 64 – 99%, preferably 75 – 99%, compared with the sequence depicted above. As it is shown below the invention discloses a lot of other homologue polypeptides which all have the biological activity of a histidine protein phosphatase.

It is a further object of the invention to provide DNA sequences which code for a polypeptide indicated above and below. Especially, the invention relates to said DNA comprising the following nucleotide sequence (SEQ. No. 1):

(ATG)GCGGTGGCGGACCTCGCTCTCATTCCTGATGTGGACATCGACTCCGACGGCGTCTTCAAG
TATGTGCTGATCCGAGTCCACTCGGCTCCCCGCTCCGGGGCTCCGGCTGCAGAGAGCAAGGAGAT
CGTGCGCGGCTACAAGTGGGCTGAGTACCATGCGGACATCTACGACAAAGTGTCGGGCGACATGC
AGAAGCAAGGCTGCGACTGTGAGTGTCTGGGCGGCGGGCGCATCTCCCACCAGAGTCAGGACAAG
AAGATTCACGTGTACGGCTATTCCATGGCCTATGGTCCTGCCCAGCACGCCATTTCAACTGAGAA
AATCAAAGCCAAGTACCCCGACTACGAGGTCACCTGGGCTAACGACGGCTAC